THE EFFECT OF CYANIDE ON RESPIRATION IN PARAMECIUM CAUDATUM AND PARAMECIUM AURELIA [1]

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In some ciliates the presence of a cytochrome-oxidase system has been established. Pitts (1932) claimed that *Colpidium campylum* showed an ititial sensitivity to HCN but that the oxygen consumption soon increased until it even surpassed normal consumption. Lwoff (1934) also found an initial inhibition followed by an acceleration in respiration in another ciliate, *Glaucoma pyriformis,* when it was exposed to KCN. Hall (1941) definitely established that HCN inhibits respiration in *Colpidium campylum* and Baker and Baumberger (1941) found that HCN inhibits respiration in *Tetrahymena geleii.*

Paramecium is usually cited as one of the several exceptions to the rule that most animal cells are sensitive to HCN. In fact, ciliates as a group have been regarded by some investigators as being insensitive to cyanide, although very few species have been tested. Lund (1918), Shoup and Boykin (1931), and Gerard and Hyman (1931) found that *Paramecium caudatum* was resistant to cyanide. However, Child (1941) refers to unpublished data obtained by Hyman, in which she found a considerable decrease in O_2 consumption of *Paramecium* in KCN. Dr. Hyman [2] has also informed the author by personal communication that she

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[2] Dr. Libbie H. Hyman has granted me the privilege of using the following communication which she sent to me at my request: "Some years ago, being skeptical of Lund's failure to find any cyanide-sensitive respiration in Paramecium, I spent a great deal of time and effort in testing the action of cyanide on the oxygen consumption of Paramecium, using Winkler's method. I met with so many difficulties that I never published the results; chief among them were the impossibility of measuring equal suspensions of Paramecium from a volumetric pipette because the animals adhere to the glass, and the toxicity to Paramecium of all waters except the culture water, which in itself has high oxygen consuming powers. However, my results indicated that starved Paramecium have no cyanide-sensitive respiration, in agreement with the finding of Lund, but non-starved ones have about 35 per cent such respiration. After giving up the work as impractical by my methods, I sought the help of Dr. Gerard. Dr. Gerard kindly consented to test the matter on his manometers but failed to find any depressing action of cyanide on non-starved Paramecium. As I played no role in this work except that I furnished the Paramecium, I feel that Dr. Gerard was over-generous in making me co-author. I was not satisfied with these results, first, because successive manometric readings were highly variable, and second, because the buffer solution used was toxic to Paramecium, depressing oxygen consumption by about 50 per cent in itself.

"As a cyanide sensitivity of the extra oxygen consumption caused by feeding was indicated in my experiments, it became interesting to know the nature of this extra respiration. I therefore attempted to compare the effects on oxygen consumption of the ingestion by Paramecium of particles without food value (carbon suspension) and of particles with food value (yeast). Here, again, I met with insuperable difficulties. I could never get any sample of yeast, no

found an inhibition of O_2 consumption in *P. caudatum* when it was exposed to HCN.

Sato and Tamiya (1937) claimed that they found cytochrome a and c in *Paramecium*. If this is true, then it is difficult to understand the insensitivity of the respiratory mechanism of this species to HCN. Because of these observations and of the unpublished results of Hyman, and since studies have not been made on the sensitivity of Paramecium to cyanide when proper KOH–KCN mixtures are used as absorption media (Krebs, 1935), the following investigation was carried out.

Material and Methods

Two species were used in this work, *Paramecium caudatum* and *Paramecium aurelia*. The culture solution used was highly buffered and was the same as was used later in the flasks of the Barcroft-Warburg apparatus for testing. The solution consisted of $K_2HPO_4.H_2O$ — 80 mg., KH_2PO_4 — 80 mg., $CaCl_2$ — 104 mg., Mg_3PO_4 — 2 mg., and redistilled water to make one liter.

In making up the stock culture, 15 gms. of timothy hay were boiled in 500 ml. of this solution for one-half hour, after which the solution was made up to its original volume by the addition of distilled water. This "broth" was then diluted further by the addition of the above buffered solution to make 4000 ml. The hydrogen ion concentration was held at pH 7.0 $\pm$ 0.2.

This culture solution, along with approximately 3 gms. of sterile hay, was put into chemical bottles with 500 ml. capacity and moderately narrow necks (3–4 cm. in diameter). About 4000 paramecia were added to each container. Within 5 days they became extremely numerous, especially in the neck region of the bottle whence they could be removed easily in large numbers.

The Barcroft-Warburg apparatus was used for ascertaining rate of oxygen consumption. The shaking mechanism was adjusted to operate at 110 complete cycles per minute. Because of the possibility of NH_3 production (Specht, 1934), a 0.3 ml. portion of 0.3 N HCl was added to the side arm (onset) of each manometer flask.

During the course of these investigations, various test solutions were made up containing different concentrations of KCN as follows: 0, 10^{-5}, 10^{-4}, and 10^{-3} M. Corresponding KOH–KCN absorption solutions were made up for each concentration of test solution according to Krebs (1935), and 0.4 ml. of the proper mixture (Pace and Belda, 1944) was added to the inner well (inset) of each flask containing organisms in KCN. To the inset of each of the flasks in which the test solution contained no KCN, a 0.4 ml. portion of M KOH was added.

A typical test was made in the following manner: Paramecia were drawn off from the top of the bottles in which they were cultured and placed in 15 ml. centrifuge tubes in which they were washed several times in fresh solution by careful centrifugation. The only time the organisms were subjected to centrifugation was

matter how many times boiled and centrifuged, that did not have high oxygen consuming powers, and all carbon suspensions also remove oxygen from the medium. However, there were indications that ingestion of a non-nutritive substance can cause as great an increase in oxygen consumption as does ingestion of food. This suggests that the extra respiration of feeding does not result from an oxidation of the food material."

during the washing process and this was carried out with great care by means of a hand centrifuge. An attempt was made to have between 2000 and 3000 *P. aurelia* or 1000 and 2000 *P. caudatum* in each 5 ml. sample. A count was always made of the organisms in each flask at the end of an experiment.

In all the tests reported here, those organisms designated "young" paramecia were taken from 5–7 day-old cultures; those designated "old" paramecia, from 15–20 day-old cultures; those designated "starved" paramecia were "old" organisms that had been placed in inorganic buffer solution without food material for 2 or 3 days. The "young" paramecia had much more food material present in the form of food vacuoles than the "old" paramecia.

Results

Effect of cyanide on respiration in Paramecium aurelia

Paramecium aurelia was the first species studied. It is a much smaller form than *P. caudatum*, but its rate of respiration per unit volume is similar to the latter (Pace and Kimura, 1944).

A number of tests were made at various KCN concentrations. Organisms that were taken from cultures 15–17 days after they had been started (i.e., "old" paramecia) were used in most of the tests. They were washed by centrifugation in the solution given above, and then divided into two portions. KCN was added to one of these portions in the concentrations designated in the table. Several tests were also carried out on starved paramecia and young paramecia. The results are presented in Table I.

P. aurelia was found to be sensitive to KCN in all the tests made, except where starved individuals were used. The normal average oxygen consumption for organisms taken from the 15 or 17 day-old cultures was 6.31 mm^3 per hour per mm^3 of cell substance at 25° C. This compares favorably with the results of Pace and Kimura (1944) who found that *P. aurelia* consumed oxygen at the rate of 6.16 mm^3 per hour per mm^3 of cell substance at 25° C.

The presence of food material may have something to do with the fact that in all the tests made, the younger paramecia showed a much greater sensitivity to cyanide than the older. In fact, starved specimens were insensitive to cyanide. When exposed to KCN at a concentration of 10^{-4} M, respiration in the young organisms was inhibited on the average by about 40 per cent. The respiration of old organisms showed an average inhibition of 22 per cent to the same concentration of KCN. At KCN concentrations of 10^{-3} M, inhibition of respiration was greater than with the lower concentration, but the results were similar insofar as young and old organisms are concerned. In young paramecia, the average O_2 consumption (1318 mm^3 O_2 per hour per million) in the buffered solution without KCN was about twice that in old organisms. An average O_2 consumption of 640 mm^3 was found for the young paramecia when they were exposed to 10^{-3} M KCN. Thus the cyanide at this concentration results in a 50 per cent inhibition in respiration in *P. aurelia*.

Effect of KCN on respiration in Paramecium caudatum

Paramecium caudatum has been studied to a much greater extent than *P. aurelia* and, as brought out previously, all the work (except for unpublished early

TABLE I

The effect of KCN on respiration in *Paramecium aurelia*. *, starved specimens; 5–7 day cultures, young specimens; all others, old specimens. Temperature, 25° C.; pH, 7.0 ± 0.2. Average volume of one million paramecia, 121.4 mm.³ (this does not include the volume of starved specimens). Each figure in columns 4 and 5 represents the average for 3 tests.

Molar concentration of KCN	Age of culture in days	Duration of test in hours	Average O_2 consumption in mm.³ per hour per million	Average O_2 consumption in mm.³ per hour per mm.³ of cell substance	Per cent inhibition
0	17*	4	462		None
10^{-4}			484		
0	16	3	746	6.14	19.9
10^{-4}			598	4.92	
0	16	3	680	5.60	29
10^{-4}			485	3.99	
0	15	5	709	5.84	36.1
10^{-4}			453	3.73	
0	15	3	808	6.65	18
10^{-4}			665	5.47	
0	15	3	841	6.92	12
10^{-4}			747	6.15	
0	7	3	906	7.46	28.5
10^{-4}			657	5.42	
0	5	5	1360	11.20	42
10^{-4}			788	6.49	
0	16*	3	520		None
10^{-3}			511		
0	15	3	818	6.73	32
10^{-3}			557	4.58	
0	5	3	1516	12.48	60
10^{-3}			605	4.98	
0	6	5	1120	9.22	40
10^{-3}			677	5.57	

results of Dr. Libbie H. Hyman) indicates that *P. caudatum* is insensitive to cyanide. One great difference in the work reported here and previous investigations carried out on the effect of cyanide on *Paramecium* is that in these experiments suitable KCN–KOH absorption mixtures rather than pure KOH were used in the manometer flasks to prevent absorption of HCN from the test solution.

The same procedures were followed here as for *P. aurelia*. The results are presented in Table II.

As indicated by the results, much variation was found in the action of KCN on *Paramecium caudatum*. In the first few tests very great difficulty was experi-

TABLE II

The effect of KCN on oxygen consumption in *Paramecium caudatum*. *, starved specimens; 5 day cultures, young specimens; all others, old specimens. Temperature, 25° C.; pH, 7.0 ± 0.2. Average volume of one million paramecia, 591 mm.^{3} Each figure in columns 4 and 5 represents the average for 3 tests.

Molar concentration of KCN	Age of culture in days	Duration of test in hours	Average O_2 consumption in mm.^{3} per hour per million	Average O_2 consumption in mm.^{3} per hour per mm.^{3} of cell substance	Per cent inhibition
0	16*	3	1565		None
10^{-5}			1518		
0	16	2	3273	5.53	15.5
10^{-5}			2734	4.62	
0	16	6	3734	6.33	15
10^{-5}			3181	5.37	
0	5	9	4420	7.47	30
10^{-5}			2650	4.48	
0	17	3	3040	5.14	None
10^{-4}			3010	5.09	
0	19	5	2700	4.56	27
10^{-4}			1978	3.34	
0	15	3	3787	6.40	40
10^{-4}			2243	3.80	
0	5	4	4270	7.22	42
10^{-4}			2475	4.18	
0	16*	5	1190		None
10^{-3}			1280		
0	15	3	3580	6.05	42
10^{-3}			2072	3.50	
0	5	12	4590	7.76	66
10^{-3}			1560	2.63	
0	15	4	4170	7.05	43
10^{-3}			2380	4.02	

enced, chiefly because some apparently minor details in manipulation were overlooked and this may have had a very noticeable effect on the results. It was suspected from the results of the first few tests that food played an important part in the degree of sensitivity of these organisms to KCN. For this reason several tests were conducted on this species under the same type of conditions as was used for *P. aurelia*, namely: (1) young paramecia (5 day cultures), (2) old paramecia (15 to 19 day cultures) and (3) starved paramecia.

The results indicate that although there was great variation in some of them, the young specimens show a greater sensitivity to KCN. The starved specimens proved to be non-sensitive. In some tests there appeared to be an actual accelera-

tion of O_2 consumption when starved *P. caudatum* was put into KCN solutions but the results may have been due to experimental error. They are not included in the table. In one test (included in table) which was made upon old organisms, there was no evidence of cyanide sensitivity; no explanation can be given for this exception.

The average inhibition of O_2 consumption found in old *P. caudatum* exposed to solutions containing 10^{-5} M KCN was approximately 15 per cent; in solutions containing 10^{-4} M, 33 per cent; and in solutions containing 10^{-3} M, 42 per cent. In young *P. caudatum* exposed to 10^{-5} M KCN, respiratory inhibition was approximately 30 per cent; in solutions containing 10^{-4} M KCN, 42 per cent; and in solutions containing 10^{-3} M, approximately 66 per cent. Thus, inhibition of oxidative metabolism increases with increase in KCN concentration, and the degree of sensitivity to cyanide seems to depend upon the quantity of food material present. This is in agreement with the results of Hyman. Higher concentrations than 10^{-3} M KCN were attempted but the results are meaningless because of such extreme variations and for this reason they have not been included in this report.

Effect of dextrose on the degree of inhibition by cyanide

Many workers have reported that one of the factors in the sensitivity of the respiratory mechanism to cyanide is the degree of carbohydrate saturation in the cell. Keilin (1932) suggests that perhaps the most important factor concerned with cellular sensitivity to cyanide is the concentration of carbohydrate. Commoner (1939) working with bakers' yeast, Emerson (1927) with *Chlorella*, and Hall (1941) with *Colpidium campylum*, all found either a greater inhibition with cyanide when dextrose was present or no inhibition without dextrose.

Since it is highly probable that a large portion of the food material of *Paramecium* is carbohydrate and since it was found that the greatest sensitivity to cyanide occurred when the greatest quantity of food was present, it was thought advisable to run respiration tests with the organisms in a dextrose solution.

Old paramecia were selected and washed in the buffered test solution containing 0.01 M dextrose. Then the solution containing the paramecia was divided

TABLE III

The effect of KCN on *Paramecium caudatum* in a 0.01M dextrose-buffer solution. All the organisms were taken from 16 to 19 day-old stock cultures. Temperature, 25° C.; pH, 7.0 ± 0.2. Average volume of one million paramecia, 580 mm.^{3} Each figure represents the average for 3 tests.

Molar concentration of KCN	Age of culture in days	Duration of test in hours	Average O_2 consumption in mm.^{3} per hour per million	Average O_2 consumption in mm.^{3} per hour per mm.^{3} of cell substance	Per cent inhibition
0	16	4	4550	7.84	48
10^{-4}			2360	4.06	
0	16	5	3860	6.65	51
10^{-4}			1890	3.25	
0	19	3	4120	7.10	54
10^{-4}			1895	3.26	

into two portions. To one portion, KCN was added to 10^{-4} M; the other portion was used as control. This experiment was repeated twice and the results are presented in Table III.

The results show that the rate of respiration in *Paramecium caudatum* is increased with the addition of dextrose to the test solution. The average rate of respiration in the dextrose-buffer solution for all tests without KCN added was 4170 mm^3 per hour per million organisms as compared to an average 3470 mm^3 in the same type of organisms tested in the buffer solution without dextrose (Table II). They also show that there was an average inhibition of 51 per cent in O_2 consumption in 10^{-4} M KCN in the dextrose-buffer solution which is much greater than the average inhibition in 10^{-4} KCN without dextrose. The average inhibition for two experiments in which the latter solution was used, was 33.5 per cent; in one of the experiments there was no inhibition whatever, but this has not been included in the average.

Discussion

Many factors may have contributed to the failure of earlier investigators to find inhibition in respiration in *Paramecium* when exposed to cyanide. Considerable error must have been caused by the absorption of free HCN by the KOH used as absorption fluid. The initial inhibitory effect followed by an increase in oxygen consumption noted in the results of Pitts (1932) and Lwoff (1934) is evidently due to the fact that little attention was given to the rapid absorption of cyanide (via distillation of HCN) by the absorption fluid. Hall (1941), using suitable KOH–KCN mixtures as absorption media, proved conclusively that respiration in *Colpidium* was cyanide sensitive.

In the investigations reported here, care was taken to prevent distillation of HCN over into the absorption fluid. However, there is another factor that may or may not have been realized by these earlier workers, namely, the food content of the paramecia with which they worked. It is possible that the organisms used by them were taken from "old" cultures and hence had comparatively little food material in them. If this be true, it explains their failure to find inhibition in respiration, for, as reported above, sensitivity seems to depend, at least partly, upon the food content of *Paramecium*. This very important factor was noted some twenty years ago by Dr. Libbie Hyman (see footnote 2).

In these experiments, the organisms were taken from the culture solution, washed, and placed in fresh test solution, and then put into manometer flasks, all within 10–15 minutes. Thus in most of the tests the organisms were actually in inorganic solution without food for 3.5 hours; in some tests 4.5–5.5 hours, but rarely longer than this. During this time, very little change could be noted in food vacuole content or size. It was also noted that respiration varied very little, if at all, from the beginning to the end of a test. In other words, the decrease in food content is so slight within this short period of time that there was no noticeable change in rate of respiration.

Carbohydrate makes up a great portion of the food of *Paramecium*. One of the most important factors in the degree of sensitivity of respiration to KCN, etc. is the concentration of carbohydrate in the cell. Thus when dextrose was added to the buffer solution in which the respiration of *Paramecium caudatum* was tested, the per cent inhibition was greater than in the buffer solution without dextrose.

Summary

1. The oxygen consumption in *Paramecium caudatum* and *Paramecium aurelia* is partially inhibited by potassium cyanide.

2. The extent of inhibition by cyanide is dependent upon the food content of the organisms as well as upon the concentration of cyanide in the solution.

3. In *P. aurelia*, starved specimens are insensitive to cyanide; old specimens are not as sensitive as young. In 10^{-4} M KCN respiration in the old organisms was inhibited by approximately 22 per cent while in the young organisms it was inhibited by approximately 40 per cent.

4. In *Paramecium caudatum*, starved specimens were non-sensitive to KCN; old specimens exposed to 10^{-3}, 10^{-4}, and 10^{-5} M KCN show, respectively, a 42, 33, and 15 per cent inhibition in respiration. Young specimens, exposed to 10^{-3}, 10^{-4}, and 10^{-5} M KCN show, respectively, a 66, 42, and 30 per cent inhibition.

5. The inhibition in the rate of respiration in *P. caudatum* was greater in buffer solution plus dextrose (0.01 M) than in the same solution without dextrose.

6. The effect of cyanide on respiration in *Paramecium* depends upon the degree of saturation of the respiratory mechanism with carbohydrate.

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